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# Methods of Determining the Oil Content of Tung Fruits and Factors Affecting It

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## INTRODUCTION

The tung industry of the United States as well as that of China and other countries is at present on a seedling-tree basis. Seedlings vary greatly in many characters, especially in tree type, in flowering habit, and in yield, size, and oil content of the fruits. In an extensive breeding program it was necessary to determine the oil content of the fruits from individual tung trees (*Aleurites fordii* Hemsl.) and the factors associated with it.

<sup>&</sup>lt;sup>1</sup>The writers are indebted to John H. Painter, horticulturist, Ernest Angelo, formerly pomologist, and other staff members of the United States Department of Agriculture Field Laboratories for Tung Investigations located at Cairo, Ga., Gainesville, Fla., and Bogalusa, La., who furnished fruit samples for oil determinations and data on tree characteristics.

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A review of the literature showed considerable variation in procedures used in preparing fruit samples and other tung products for analysis and in determining their oil and moisture contents; little information on sampling or on the association of external tree characteristics with oil content of the fruits was found. Therefore, preliminary experiments were made to determine: (1) A satisfactory method of preparing a sample of tung fruits and determining their moisture and oil contents; (2) the variation in oil content of mature fruits of individual tung trees and in the number of fruits required to obtain a sample representative of a tree or a plot; and (3) the association of external factors or tree characteristics with the oil content of the fruits.

# REVIEW OF LITERATURE

Several methods of preparing kernels and other products of tung for oil extraction have been used. In 1936 Pollard and Ellis  $(12)^2$ reported a method of extraction of oil from press cake and meal.<sup>3</sup> The press cake was crushed in a mortar, a 25- to 40-gm. sample was transferred to a Soxhlet apparatus, and the entire system including the flask was swept out with nitrogen. The sample was extracted for 10 hours, and the solvent was evaporated from the flasks in the presence of nitrogen on a steam bath. The oil was determined by weighing the residue. Oil was extracted from the meal in the same manner except that the meal was sifted through a 14-mesh sieve.

In 1936 McKinney and Jamieson (10) published a method for extraction of oil from tung kernels. They determined the average weight of fruits in each sample as well as the percentages of nuts and of kernels. In order to obtain a representative sample, it was necessary to grind about 60 gm. of kernels to a meal that would pass a 6mesh sieve. The sample was thoroughly mixed, and the oil content was determined by extraction with petroleum ether in somewhat the same way as in the determination of oil in cottonseed (15); the sample was reground in a mortar after the first extraction, and this process was repeated four times.

Pickett and Brown (11) in 1938 used approximately 25 fruits for each sample and dried them at room temperature. The fruits were divided into their component parts, and the kernels were prepared for analysis by means of a nutmeg grater. The samples were extracted for 16 hours with a residue casing-head gasoline boiling below 100° C. The solvent was removed on a water bath, and the oil was then dried to constant weight in an oven at 105° in an atmosphere of carbon dioxide. Moisture content of the grated material was determined by drying at 100° for 5 hours.

In 1939 McKinney and Freeman  $(\mathcal{P})$  reported a modification of their method. They placed 80 fruits in a closed 2-gallon lard can and found that after 1 week the moisture was equally distributed among individual fruits. Two subsamples of 10 fruits each were taken at random for the analyses. The fruits in the samples were separated into their component parts—hull, shell, and kernel. Mois-

<sup>&</sup>lt;sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 24.

<sup>&</sup>lt;sup>3</sup>The meal consists of tung kernels with some shells as ground at the tung mill for commercial processing. Press cake is the residue after the expressing of the oil.

ture in these components was determined by drying at 101° to 102° C. for 24 hours. Oil was determined by grinding the kernels twice in a household food grinder and extracting a 5-gm. sample with petroleum ether in a Butt extraction tube fitted with a condenser and tared flask. After 4 hours the residue was ground with sand and re-extracted for an additional 2 hours. This treatment was reported to remove the oil completely. The solvent was removed and the oil dried at 100° with 28 to 29 inches of vacuum for 40 minutes.

The literature shows that different types of extraction apparatus and solvents and extraction periods of various lengths have been used by the various investigators. Pollard and Ellis (12), realizing the highly reactive properties of tung oil, attempted to minimize oxidation by extracting in an atmosphere of nitrogen. François (3) and Pickett and Brown (11) dried the oil under carbon dioxide for the same reason. Different temperatures and drying methods have been used by the several investigators in determining the percentage of moisture in the kernels.

A review of the literature shows that few attempts have been made to determine the variation existing among individual fruits and the number of tung fruits required for an adequate sample (5).

## MATERIAL AND EQUIPMENT

The mature tung fruits used in the preliminary experiments on methods were obtained near Gainesville. Fla., from commercial orchards and from orchards of the Florida Agricultural Experiment Station. All fruits were apparently normal or average fruits obtained from trees in a good condition of growth and productivity. Samples for the study of factors affecting the oil content of tung fruits were taken in orchards throughout the tung belt.

A Goldfisch extraction unit was used to extract the oil, since it effects rapid condensation of the solvent, thereby accelerating extraction and surrounding the kernel material with solvent vapors, which minimize oxidation.

# DETERMINATION OF MOISTURE IN TUNG FRUITS

To bring the fruits to an approximately standard moisture content (about 10 percent), Potter and associates (13) brought them into equilibrium with the vapor pressure of a saturated solution of calcium chloride at a room temperature of about 25° C. In this circular fruits brought to equilibrium in this way are referred to as "equalized." Advantages of the method are that the degree of dryness obtained approximates the optimum for milling tung fruits and that data based on such equilibrium are easily interpreted by growers and mill operators.

One hundred fruits were placed in a chamber for 4 days and brought into approximate equilibrium with the vapor pressure of a saturated solution of calcium chloride at room temperature. They were then placed in a tightly covered tin container, according to the method reported by McKinney and Freeman  $(\mathcal{P})$ , and left for a week in order to insure uniformity in moisture content; afterward their moisture was determined by several methods.

#### Whole and Ground Fruits

Twenty-five fruits were weighed individually and dried to constant weight in a vacuum oven over phosphorus pentoxide at 100° C. and 4-mm. pressure for successive 4-hour periods. This was done because the oil oxidizes and polymerizes rapidly in the air at 100° and at normal pressure. The time required for whole individual fruits dried under these conditions to attain constant weight ranged from 12 to 20 hours. In spite of the precautions taken to bring the fruits to equilibrium, moisture in the individual fruits ranged from 8.71 to 10.78 percent, with a mean of  $9.77 \pm 0.11$  percent.

To study the effect of grinding on the rate of the loss of moisture from tung fruits during the drying process, 25 fruits from the lot of 100 were ground 4 individually in a Wiley mill to pass a 2-mm. sieve. Two 5-gm. samples of each fruit thus ground were then dried under the conditions described for whole fruits. Constant weight was attained at the end of 4 hours; the moisture content of individual fruits ranged from 8.19 to 11.70 percent, with a mean of  $9.60 \pm 0.20$  percent. This does not differ significantly from  $9.77 \pm 0.11$  percent obtained by the previous method. The important difference is the much shorter period required to remove the moisture from the ground material, 4 hours as compared with 12 to 20 hours. A composite sample was obtained by grinding 25 other fruits of the same lot of 100 in a Wiley mill. After a thorough mixing triplicate 5-gm. samples were dried as before. Constant weight was attained at the end of 4 hours; the amount of moisture found by this rapid, simple method was 9.59 percent, which agrees very closely with that found by the 2 previous methods.

## Components of Fruits

Twenty-five of the original 100 equalized fruits were separated into their component parts and were found to consist of 41.5 percent outer hull, 8.3 percent inner hull, 20.8 percent shell, and 29.4 percent kernel. Each component was divided into 2 approximately equal samples, 1 of which was ground in a Wiley mill to pass a 2-mm. sieve. The moisture in the unground and ground samples was determined by drying to constant weight in a vacuum oven over phosphorus pentoxide at 100° C. and 4-mm. pressure. Grinding facilitated drying of all components except the shell (table 1). It was more difficult to remove the moisture from unground than from ground kernels. They yielded on the average 3.08 percent moisture when not ground as compared with 4.33 percent when ground. Otherwise the moisture determinations of the unground and ground components agreed rather well.

The wide variation in moisture content of the different parts of the tung fruit is of considerable interest. For example, after the parts of fruits had had ample opportunity to come to equilibrium, the kernel contained 4.33 percent of moisture and the outer hull 14.06 percent. Although the kernels constituted 29.4 percent of the total weight of the whole fruits, they contained only 12.78 percent of the total moisture, whereas the inner and outer hulls, which constituted

<sup>&</sup>lt;sup>4</sup> In this circular material prepared in a Wiley mill is referred to as "ground," although it is recognized that the mill operates by a cutting action.

49.8 percent of the total weight of the equalized fruits, contained 67.29 percent of the total moisture.

TABLE 1.—Moisture content of components of unground and ground 1tung fruits as determined by drying over phosphorus pentoxide at100° C. and 4-mm. pressure

Portion or com- ponent	Mean weight per	Propor- tion of total	Drying period for bringing material to constant weight		eriod ging l to veight Moisture content of component		Proportion of total moisture in whole fruits	
	fruit weight		Un- ground	Ground	Un- ground	Ground	Un- ground	Ground
Outer hull Inner hull Shell Kernel Whole fruits (cal- culated)	Grams 11. 0 2. 2 5. 5 7. 8 26. 5	Percent 41. 5 8. 3 20. 8 29. 4 100. 0	Hours 8 8 4 8	Hours 4 4 4 4	Percent 14. 44 10. 60 9. 90 3. 08 9. 81	Percent 14. 06 10. 83 9. 71 4. 33 10. 04	Percent 61. 15 8. 85 20. 77 9. 23 100. 0	Percent 58. 27 9. 02 19. 93 12. 78 100. 0

<sup>1</sup> Prepared in Wiley mill, which actually cuts rather than grinds.

Essentially the same results were obtained by the several methods tested for determining moisture. By using the standard deviation 0.55, from which the standard error of the mean moisture content of the 25-fruit sample was calculated (p. 4), it can be estimated that, if the mean moisture content of the sample were calculated from a determination on a single fruit, the 5-percent fiducial limits of percentage moisture would be 8.63 to 10.91; from a 2-fruit sample, 8.97 to 10.57; from a 4-fruit sample, 9.20 to 10.34; and from a 10-fruit sample, 9.41 to 10.13. It must be remembered that all the fruits used in these tests had previously been brought to approximate equilibrium over a saturated solution of calcium chloride at room temperature. Fruits taken direct from the orchard would be expected to be much more variable in moisture content. Duplicate determinations on 5-gm. portions from a composite sample of 25 ground fruits would give the average moisture content far more precisely than any sample of whole fruit that would require equivalent space in a drying oven. This method appears to be the most rapid and satisfactory.

#### FLAKED KERNELS

It was evident that any grinding process used on tung kernels containing 50 to 70 percent of oil would result in the expression of some of the oil. It is possible that some of the liberated oil would adhere to all surfaces with which it came in contact and thus the apparent content of oil, moisture, and other constituents might be materially altered. To overcome similar difficulties in the analysis of pecan kernels, Crane and Hardy (2) sliced them into very thin flakes by means of an inverted jack plane. Tung kernels were prepared for moisture and oil determinations by slicing them longitudinally into flakes  $50\mu$  to  $100\mu$  in thickness, and the moisture content was determined by several methods.

Because of oxidation of tung oil it was found impossible to determine the moisture in the flaked kernels in air at normal pressure at 100° C. (table 2). Mature kernels can be safely dried, however, in a vacuum oven for 8 hours at 100° and 4-mm. pressure over phosphorus pentoxide, but this temperature is too high for immature kernels or for materials in which carbohydrates are later to be determined. The percentages of moisture in the kernels obtained at 70° are comparable with those obtained at 100°, and they agree well with the Dean–Starke distillation method (1). This material, dried at 70°, may still be used for carbohydrate analysis.

 TABLE 2.—Moisture in flaked tung kernels as determined on duplicate

 10-gm. samples by several methods

	D	Distilled with xylene (Dean-Starke distillation method)		
Sample	100° C. and normal pressure100° C. and 4-mm. pressure70° C. and 4-mm. pressure			
1 2	(1) (1)	Percent 4. 8 4. 9	Percent 4. 8 4. 8	Percent 5. 0 4. 8

<sup>1</sup> Sample gained weight.

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# DETERMINATION OF OIL IN TUNG KERNELS

#### BUTT TUBE VERSUS GOLDFISCH EXTRACTOR

In an attempt to minimize expression and loss of oil, a sample of 100 kernels was prepared in a Wiley mill. The material was thoroughly mixed, and moisture determinations were run on two 5-gm. samples by placing them in a vacuum oven for 8 hours at 100° C. and 4-mm. pressure over phosphorus pentoxide. Oil was determined in 4 additional samples of this same material by extracting with petroleum ether—2 in a Butt tube extraction unit and 2 in the Goldfisch unit. After 4 hours all were ground with sand and then reextracted for an additional 2 hours, and the weight of oil was determined after drying on a steam bath for 30 minutes and finally in a vacuum oven over phosphorus pentoxide at 70° and 4-mm. pressure. The data from the two extraction units are almost identical (table 3). However, continued extraction of all the samples in the Goldfisch unit for an additional 18-hour period removed a mean of 2.57 percent more oil.

#### GRINDING VERSUS FLAKING

Since Crane and Hardy (2) obtained the best results in the extraction of oil from pecan kernels by thinly slicing them on an inverted  

 TABLE 3.—Comparison of the efficiency of the Butt tube and the Goldfisch methods of oil extraction on four 5-gm. samples of flaked tung kernels

	Oil extracte and b					
Sample	First 6	hours <sup>1</sup>	18 additional	Total oil	Mean oil	
-	Butt tube	Goldfisch unit	hours (Goldfisch)			
1 2 3 4	Percent 59. 65 59. 84	Percent 59. 84 59. 97	Percent 2, 19 2, 34 3, 12 2, 63	Percent 61. 84 62. 18 62. 96 62. 60	Percent           62.01           62.78	

[Percentage figures based on original dry weights]

<sup>1</sup> All samples ground with sand after 4 hours and then re-extracted 2 hours more.

jack plane, this method was compared with grinding. In slicing, the kernel is subjected to only slight pressure and should, therefore, lose only a negligible quantity of oil. Accordingly, of 100 kernels taken at random, 50 were flaked longitudinally to a thickness of  $50\mu$  to  $100\mu$  on an inverted jack plane and 50 were ground in the Wiley mill to pass a 2-mm. sieve. Four 5-gm. samples of the flaked material and 4 of the ground were weighed and extracted for 4 hours in the Goldfisch unit; then 2 of the ground samples and 2 of the flaked were ground in a mortar with 1 gm. of sand. The samples were returned to the extractor, and the oil was determined at the end of 12-, 18-, and 24-hour extraction periods (table 4).

 

 TABLE 4.—Comparison of the rate at which oil is extracted with a Goldfisch unit from flaked and ground tung kernels and the effect of grinding with sand after 12-, 18-, and 24-hour extraction periods

		Oil extracted during-					
Sample Preparation of kernel	First 12 hours	Next 6 hours	First 18 hours (total)	6 addi- tional hours	24 hours (total)	oil of dupli- cates	
1 2 3 4	Flaked; not "reground"_ do Flaked; "reground" do	$\begin{array}{c} Pct. \\ 64. \ 17 \\ 64. \ 15 \\ 64. \ 21 \\ 64. \ 25 \end{array}$	$\begin{array}{c} Pct. \\ 0.\ 27 \\ .\ 29 \\ .\ 15 \\ .\ 10 \end{array}$	$\begin{array}{c} Pct. \\ 64. \ 44 \\ 64. \ 44 \\ 64. \ 36 \\ 64. \ 35 \end{array}$	$\begin{array}{c} Pct. \\ 0.\ 17 \\ .\ 19 \\ .\ 04 \\ .\ 06 \end{array}$	$\begin{array}{c} Pct. \\ 64. \ 61 \\ 64. \ 63 \\ 64. \ 40 \\ 64. \ 41 \end{array}$	$Pct. \\ 64. 62 \\ 64. 41 \end{cases}$
5 6 7 8	Ground; not reground Ground; reground Ground; reground	$58.89 \\ 58.83 \\ 61.17 \\ 61.25$	$\begin{array}{c} 2.\ 06\\ 2.\ 02\\ 1.\ 58\\ 1.\ 58\end{array}$	$\begin{array}{c} 60. \ 95 \\ 60. \ 85 \\ 62. \ 75 \\ 62. \ 83 \end{array}$	$     . 82 \\     . 93 \\     . 59 \\     . 42   $	$\begin{array}{c} 61.\ 77\\ 61.\ 78\\ 63.\ 34\\ 63.\ 25 \end{array}$	$\Big\} 61.78 \\ \Big\} 63.30$

[Percentage figures based on original dry weights]

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Regrinding with sand increased the quantity of oil extracted from kernels ground in the Wiley mill. As there was a tendency for the ground material to be compacted into little balls during the grinding process, such regrinding aided in the removal of the oil by breaking up these lumps. McKinney and Freeman ( $\vartheta$ ) reported similar results on kernels ground in a household food grinder. In the present experiment "regrinding" with sand did not seem to increase the amount of oil extracted from flaked kernels. More extensive tests indicated that fully 99.0 percent of the total oil in flaked tung kernels of a rather uniform thickness of  $50\mu$  can be extracted in 12 hours in the Goldfisch extractor without grinding (table 5). As a result of these studies all kernels for oil determinations at the writers' laboratories are prepared with a power-driven mechanical flaker.

 

 TABLE 5.—Cumulative percentages of total oil removed from flaked tung kernels during successive extraction periods

	Oil extracted from indicated sample						
Hours of extraction	1	2	3	4	5	6	
$\begin{array}{c} 6 \\ - \\ 7 \\ - \\ 8 \\ - \\ 9 \\ - \\ 10 \\ - \\ $	Percent 97. 72 98. 06 98. 70 99. 07 99. 13 99. 20 99. 34 99. 45 99. 49 99. 57 99. 86 100. 00 100. 00	$\begin{array}{c} Percent\\ 97.\ 67\\ 98.\ 04\\ 98.\ 65\\ 98.\ 82\\ 98.\ 91\\ 99.\ 02\\ 99.\ 23\\ 99.\ 39\\ 99.\ 40\\ 99.\ 48\\ 99.\ 83\\ 100.\ 00\\ 100.\ 00\\ \end{array}$	$\begin{array}{c} Percent\\ 98. 10\\ 98. 42\\ 98. 94\\ 99. 11\\ 99. 19\\ 99. 26\\ 99. 40\\ 99. 49\\ 99. 51\\ 99. 57\\ 99. 86\\ 100. 00\\ 100. 00\\ \end{array}$	$\begin{array}{c} Percent\\ 97.\ 15\\ 97.\ 65\\ 98.\ 43\\ 98.\ 75\\ 99.\ 15\\ 99.\ 23\\ 99.\ 42\\ 99.\ 42\\ 99.\ 42\\ 99.\ 43\\ 99.\ 49\\ 99.\ 85\\ 100.\ 00\\ 100.\ 00\\ \end{array}$	Percent 97. 86 98. 21 98. 94 99. 09 99. 18 99. 28 99. 40 99. 51 99. 52 99. 60 99. 86 99. 98 100. 00	Percent 98. 07 98. 41 99. 03 99. 17 99. 21 99. 31 99. 43 99. 43 99. 49 99. 51 99. 57 99. 88 100. 00 100. 00	

[Percentage figures based on original dry weights]

#### DISTRIBUTION OF OIL IN KERNELS

If the kernel is of uniform composition throughout, the most representative sample of a lot of fruit would be obtained by using a small portion of each of a large number of kernels. To determine whether different parts of the kernel vary in oil content, the micropylar ends and the midsections of kernels from six different seedling trees were flaked and sampled separately. In every comparison the micropylar ends were lower in oil content than the midsections, the difference ranging from 1.63 to 4.48 percent, with a mean of 2.64 percent. Hence, all later samples for oil analysis consisted of halves of kernels which were flaked longitudinally.

#### TIME REQUIRED FOR EXTRACTION OF OIL

To determine the time required for the extraction of the oil from flaked kernels in the Goldfisch unit, six 5-gm. samples were extracted with petroleum ether for successive periods until no additional oil was removed. The samples were first extracted for 6 hours, the oil was then weighed, and the samples were re-extracted in the same manner for nine successive periods of 1 hour each and then for three periods of 3 hours each. The additional increments of oil removed during each period were determined (table 5). The extraction of oil from all six samples proceeded quite uniformly throughout the study.

Over 97 percent of the oil extractable by this method was removed during the first 6 hours and over 99 percent by the end of 11 hours. Since the percentage of the total oil extracted from all the samples during the first 12 hours ranged from 99.23 to 99.43 percent, with a mean of 99.35, one can estimate the total amount of oil extractable from any particular sample by multiplying the weight of oil extracted at the end of 12 hours by the factor  $100 \div 99.35 = 1.00654$ . The 12-hour period has been adopted at the laboratories with which the writers are associated as standard for extraction of all samples of flaked tung kernels. For comparative purposes, for example, in evaluating certain trees as potential breeding material, the use of the factor is not essential.

# DRYING AND WEIGHING OF OIL

The weight of oil can be determined directly or by getting the difference in weight of the kernel sample before and after extraction. In the method of direct weighing precautions are necessary to prevent spattering and oxidation of the oil during drying. It is more convenient to dry the thimble of extracted residue first in air for 30 minutes and then in a vacuum oven at 70° C. and 4-mm. pressure over phosphorus pentoxide for 4 hours and then to weigh it than it is to free the oil of solvent and then weigh the oil. Rapid weighing of the residue is essential because of its hygroscopic properties.

If oven-dry thimbles are used, the loss in weight during extraction is due to oil extracted and moisture driven off from the sample of tung kernel; hence, a correction must be made for moisture content of the sample. To compare the two methods, a sample of 100 kernels from tree A-12 was flaked and thoroughly mixed and oil was determined in twelve 5-gm. subsamples by both methods. After being extracted in the Goldfisch apparatus for 12 hours, the residual meal was ground to about 60-mesh size in an intermediate Wiley mill, with approximately 90-percent recovery. The reground meal was extracted for another 4 hours; the additional oil was weighed and corrected for loss in recovery of the subsample and then the total oil was calculated as percentage of the original subsample (table 6).

The values obtained by direct weighing of the oil are on the average  $0.42 \pm 0.21$  percent lower than those calculated from loss in weight of the subsample, and the standard deviation of the determinations made by weighing the oil is slightly lower than that of the determinations calculated from loss in weight of the subsample. As weighing the oil is not significantly the more precise method, the simpler and more expeditious method of weighing the residue will be found adequate for most purposes. It is to be noted in the data for total oil, as

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TABLE 6.—Cumulative percentages of oil in 12 subsamples from 1 sample of flaked tung kernels from tree A-12 as determined (1) directly by weight of oil and (2) indirectly by loss in weight of the subsample during extraction <sup>1</sup>

		Oil content determined—						
	Subsample	By weight period	of oil after l of—	Indirectly by loss in weight of subsample after period of—				
		4 additional hours	16 hours (total)	4 additional hours	16 hours (total)			
1 2 3 4 5 6 7 8 9 10 11 12		$\begin{array}{r} Percent \\ 0.59 \\ .47 \\ .58 \\ .81 \\ .42 \\ .41 \\ .53 \\ .61 \\ .50 \\ .59 \\ .55 \\ .51 \end{array}$	$\begin{array}{c} Percent \\ 60, 79 \\ 61, 72 \\ 60, 94 \\ 60, 83 \\ 60, 95 \\ 61, 84 \\ 61, 68 \\ 61, 77 \\ 60, 97 \\ 61, 24 \\ 61, 95 \\ 61, 39 \end{array}$	$\begin{array}{r} Percent \\ 0.08 \\ .65 \\ .23 \\ 1.32 \\ .23 \\ .70 \\ .56 \\ .11 \\ .32 \\ .00 \\ .17 \\ .23 \end{array}$	$\begin{array}{c} Percent \\ 60, 89 \\ 62, 38 \\ 60, 89 \\ 61, 48 \\ 61, 67 \\ 62, 51 \\ 62, 02 \\ 61, 71 \\ 61, 90 \\ 61, 23 \\ 62, 80 \\ 61, 60 \end{array}$			
	Mean Standard devi- ation		61. 34 . 44		61. 76 . 60			

<sup>1</sup> Data furnished by S. G. Gilbert, Division of Fruit and Vegetable Crops and Diseases. At end of the 12-hour extraction the sample was reground to pass a 60-mesh screen in an intermediate Wiley mill, with 10-percent loss. The meal was then re-extracted for 4 additional hours. The additional oil and loss of weight on extraction were corrected for the percentage of meal lost in grinding.

determined by weighing the oil after re-extraction, that there was a considerable difference between subsamples; the percentages ranged from 60.79 to 61.95, a difference of 1.16 percent. As determined by the indirect method, they ranged from 60.89 to 62.80 percent, a difference of 1.91. It is believed that there is a considerable error of sampling in drawing successive subsamples from the same lot of flaked kernels and that other uncontrolled factors may influence the results to a considerable extent. The percentage of oil in the dry kernels or in whole fruits can be calculated by the use of the following formulas:

(D Oil in kornel (percent)	ry weight of kernel sample — weight of ex- tracted residue) (100.654)
on m kerner (percent) =	Dry weight of kernel sample
Oil in kernel (percent) $= \frac{(}{Dr}$	Weight of oil) (100.654) y weight of kernel sample
(Pe Oil in fruits (percent) =	rcentage of oil in kernel) (percentage of kernel in whole fruits) 100

#### EXTRACTION OF OIL BY DIFFERENT SOLVENTS

The percentages of oil extracted from flaked tung kernels by a number of solvents were compared. The quality of the oil was evaluated by the iodine value, saponification value, diene value, specific gravity, and refractive index. The kernels were removed from 100 fruits from a single tree and half of each kernel was flaked longitudinally on an inverted jack plane. After a thorough mixing 5-gm. subsamples of the flaked kernel were placed in a Goldfisch extraction unit and extracted with each of 5 solvents (table 7).

Practically identical results were obtained with acetone. ethyl ether, and petroleum ether. The physical constants of the oil extracted by these solvents agreed very closely. Trichloroethylene gave a slightly lower oil content. The oil extracted with trichloroethylene had somewhat higher specific gravity than that extracted with benzene, which in turn had slightly higher specific gravity than oil extracted with acetone, ethyl ether, or petroleum ether. The refractive index of oil extracted with benzene was almost identical with that of oil extracted with acetone, ethyl ether, or petroleum ether, but that of oil extracted with trichloroethylene was relatively low. There is a tendency for acetone and ethyl ether to remove small quantities of sugar from rather moist tung kernels. Since acetone is miscible with water, it will penetrate immature tissues better than either ethyl ether or petroleum ether. For kernels having a moisture content that does not exceed 5 percent, the extraction of nonoily material by any of these solvents is negligible.

Petroleum ether (low-boiling) has been used in most of the standard procedures for oil analysis. It was employed by the writers in routine analytical work since none of the other solvents proved to extract more or better oil.

# DETERMINATION OF SIZE OF ADEQUATE SAMPLES

To determine how many fruits are required to make an adequate sample, it was necessary to estimate the variation in the oil content of individual fruits produced on the same tree. Having this information, one can determine by means of statistical procedures the degree of precision attained by using any given number of individual fruits per sample. The oil content was determined individually for each of 16 fruits taken at random from each of 8 trees (table 8).

That the fruits of some trees are much more variable than those of others is indicated by the standard deviations shown in table 8. The fruits from trees 4, 6, and 8 showed the least variation, but even their ranges from lowest to highest in percentage of oil in the individual fruits were considerable. In the case of tree 4 the percentage of oil in individual fruits ranged from 21.2 to 28.6. The mean oil content of the fruits from tree 2, as determined from a sample of 16 fruits, has, according to Snedecor (14), a standard error of 0.70 percent. The standard error of the difference of two such means, according to Snedecor (14), would be 0.99 percent. Since a statistically significant difference between two means must be approximately twice its standard error, it follows that, on the basis of samples of 16 fruits each, one could distinguish between the oil content of the fruits of 2 trees provided that (1) the fruits of both types have the same degree

12TABLE 7.-Comparison of various fat solvents as to percentage and quality of oil extracted from a homogeneous sample of

flaked tung k*érnels* 

[Percentage figures based on original dry weights]

 $\begin{array}{c} 1.5170\\ 1.5170\\ 1.5170\\ 1.5168\\ 1.5168\end{array}$ 1.5161 Refraeindex<sup>3</sup> tive gravity<sup>3</sup> Specific 9365 9344 0.9330. 9329 . 9331  $\begin{array}{c} 68.\ 68\pm 0.\ 60\\ 68.\ 94\pm .\ 06\\ 67.\ 91\pm .\ 90\\ 66.\ 40\pm .\ 94\\ 69.\ 80\pm 1.\ 05 \end{array}$ Mean value Diene value<sup>2</sup> Constant of extracted oil Number 00 Determinations  $\begin{array}{c} 193. \ 6\pm 0. \ 81\\ 193. \ 4\pm \ . \ 63\\ 193. \ 2\pm \ . \ 36\\ 192. \ 4\pm \ . \ 59\\ 192. \ 4\pm \ . \ 59\\ 191. \ 9\pm \ . \ 74\end{array}$ Mean value Suponification value Numbermina-Detertions  $\begin{array}{c} 161. \, 6\pm 0. \, 76\\ 161. \, 6\pm . \, 22\\ 161. \, 7\pm . \, 58\\ 160. \, 8\pm . \, 36\\ 160. \, 2\pm . \, 75\\ 161. \, 2\pm . \, 75\\ \end{array}$ Mean value Iodine value<sup>1</sup> Number Deter-00 00 00 <del>4</del> minations  $\begin{array}{c} 67.\ 59\pm0.\ 23\\ 67.\ 40\pm \ 29\\ 67.\ 45\pm \ 12\\ 67.\ 20\pm \ 31\\ 66.\ 59\pm \ 20\end{array}$ Mean value Percent Oil content Number 804208 Determinations Petroleum ether ---Trichloroethylene. Solvent Ethyl ether\_ Acetone\_\_ Benzene\_.

Rosenmund-Kuhnhenn method (6, p. 96).

<sup>2</sup> Ellis-Jones method (7, p, 399).

3 determinations per solvent with no deviation from value given.

#### TABLE 8.—Oil content of 16 individual whole fruits from each of 8 seedling tung trees

[Percentage figures based on original dry weights]

EXPERIMENTAL DATA

		Oil content of indicated tree						
Fruit	1	2	3	4	5	6	7	8
1 2 3 4 5 6 7 8 9 10 10 11 12 13 14 15 16 Standard deviation	$\begin{array}{c} Per-\\ cent\\ 22, 1\\ 21, 0\\ 22, 5\\ 22, 4\\ 18, 1\\ 19, 6\\ 18, 0\\ 19, 6\\ 16, 9\\ 21, 2\\ 26, 6\\ 26, 3\\ 15, 0\\ 24, 0\\ 17, 9\\ 23, 0\\ 3, 3\\ \end{array}$	$\begin{array}{c} Per-\\ cent\\ 24.\ 4\\ 22.\ 9\\ 27.\ 9\\ 17.\ 8\\ 23.\ 7\\ 25.\ 3\\ 22.\ 7\\ 26.\ 3\\ 26.\ 4\\ 19.\ 0\\ 22.\ 7\\ 23.\ 7\\ 23.\ 5\\ 26.\ 4\\ 23.\ 5\\ 26.\ 4\\ 2.\ 8\end{array}$	$\begin{array}{c} Per-\\ cent\\ 24.8\\ 23.5\\ 28.5\\ 29.9\\ 22.3\\ 20.3\\ 27.7\\ 4\\ 27.8\\ 26.7\\ 25.7\\ 21.5\\ 30.3\\ 27.2\\ 18.2\\ 4.0 \end{array}$	$\begin{array}{c} Per-\\ cent\\ 24.1\\ 23.5\\ 21.2\\ 27.2\\ 8.6\\ 24.1\\ 22.1\\ 26.7\\ 26.7\\ 26.7\\ 25.2\\ 27.1\\ 24.8\\ 26.3\\ 23.8\\ 27.1\\ 2.0\\ \end{array}$	$\begin{array}{c} Per-\\ cent\\ 26,2\\ 31,2\\ 28,2\\ 30,6\\ 29,8\\ 30,6\\ 24,4\\ 31,6\\ 30,4\\ 31,6\\ 30,4\\ 31,8\\ 31,6\\ 30,4\\ 31,8\\ 31,6\\ 30,9\\ 27,8\\ 30,9\\ 2,2\\ \end{array}$	$\begin{array}{c} Per-\\cent\\24.\ 7\\24.\ 5\\22.\ 9\\25.\ 8\\20.\ 1\\24.\ 5\\23.\ 1\\24.\ 4\\26.\ 4\\23.\ 1\\26.\ 6\\23.\ 2\\24.\ 2\\23.\ 2\\26.\ 4\\21.\ 9\\1.\ 8\end{array}$	$\begin{array}{c} Per-\\ cent\\ 31, 3\\ 19, 5\\ 31, 4\\ 31, 2\\ 31, 4\\ 33, 0\\ 27, 6\\ 30, 1\\ 23, 8\\ 30, 9\\ 32, 4\\ 30, 9\\ 32, 7\\ 33, 2\\ 24, 2\\ 4, 1\\ \end{array}$	$\begin{array}{c} Per-\\ cent\\ 29, 3\\ 29, 5\\ 24, 4\\ 27, 7\\ 27, 2\\ 26, 0\\ 30, 2\\ 27, 2\\ 29, 2\\ 27, 4\\ 28, 1\\ 29, 0\\ 27, 6\\ 28, 6\\ 30, 2\\ 29, 6\\ 1, 6\\ \end{array}$
Fruits needed per sample 1	Num- ber 22	Num- ber 16	Num- ber 33	Num- ber 8	Num- ber 10	Num- ber 7	Num- ber 34	Num- ber 5

#### ANALYSIS OF VARIANCE

Source of variation	Sum of squares	Degrees of freedom	Variance
Total Between trees Within trees	2, 111. 31 1, 062. 52 1, 048. 79	$\begin{array}{c}127\\7\\120\end{array}$	8. 740

<sup>1</sup> Number required to obtain a value for the mean oil content that will have a standard error of approximately 0.70.

of variability as the fruits of tree 2 and (2) the actual mean difference in oil content is not less than 2.00 percent.

For trees having more variable fruits, larger samples would be required to attain the same degree of precision. The number required for each of the other 7 trees was determined by Snedecor's formula (14).<sup>5</sup> The desired value for the standard error of the mean, 0.70 per-

 ${}_{5} \frac{S}{X} = \frac{s}{\sqrt{n}}$ 

where  $s_{\overline{x}} = \text{standard error of the mean}$ 

s =standard deviation of population n =number in sample.

cent, and the appropriate standard deviation for fruits of each tree were substituted in the equation and it was solved for n, the number of fruits per sample (table 8). For example, in the case of trees having fruits of the same degree of variability as those of tree 7, 34 fruits would be required per sample to attain the same degree of precision that may be had with a 5-fruit sample from tree 8.

In practice it is impossible to determine the variability of the fruits on each of hundreds of individual trees; therefore, it is necessary to use a sample of such size that oil content of the fruits of all or nearly all trees will be determined with reasonable precision. A pooled standard deviation (2.96) for all 8 trees was calculated from the analysis of variance shown in table 8. It was found that on the average a sample of 16 or 17 fruits would give the desired precision, and a 25fruit sample was adopted as the standard. This number will insure reasonable precision and at the same time keep the labor of sampling and analysis within practicable limits.

To determine how the 25-fruit sample works out in practice, one may study its application to the 8 trees of known variability. Twentyeight possible comparisons can be made among these trees—tree 1 with tree 2, tree 1 with tree 3, and so forth. The standard error of the difference between the means of trees 3 and 7 is 1.14 percent, and therefore the least difference between the fruits of these 2 trees significant at the 0.05 level is 2.35 percent. Similarly, on the basis of 25-fruit samples the least significant difference between trees 1 and 7 would be 2.17 percent; between trees 1 and 3, 2.15 percent; between trees 2 and 7, 2.04 percent; and between trees 2 and 3, 2.02 percent. In all of the 23 other possible comparisons the least significant difference is less than 2 percent. The precision attained by using the 25-fruit samples seems adequate for making initial selections of tung trees for breeding purposes.

### FACTORS AFFECTING OIL CONTENT OF TUNG FRUITS

## ANNUAL VARIATIONS

The work on selection was begun in 1938, a year of heavy crop; 221 trees were selected that year. The primary basis of selection was productivity; other factors considered were size, type, and bearing habits of the tree, size and thickness of hull, season of maturity of the fruits, and degree of filling of the nuts. In 1939 a severe late-February freeze practically destroyed the crop and selections were made largely for frost resistance. Very few of the original 1938 selections bore fruit, and samples analyzed in 1939 came almost entirely from a new group of trees. In 1940 another heavy crop was borne. Samples from a number of additional new selections and also from some of the most productive of the 1938 and 1939 selections were analyzed.

When selections are made in the orchard, it is impossible to know the oil content of the fruit, except that in general good oil content may be expected in thin-hulled fruits that have well-filled nuts. No trees with thick-hulled fruits were selected. Since a relatively large group of trees was studied each year, it was reasonable to expect that the mean percentage of oil in the three groups of trees would be approximately the same, unless there was some significant yearly effect. In other words, the number of trees selected each year was large enough to level out inherent genetic differences in potential oil content.

Frequency distributions of the samples for each year, classified according to oil content of the fruits, are shown (table 9). Over the 3-year period the oil content of whole, equalized fruits of selected trees ranged from 6 to 28 percent. The class of greatest frequency was 20 percent, and the mean oil content for all samples was 20.3 percent. Analyses of fruits from experimental plots in commercial orchards usually show a mean of about 19.5 to 20 percent. This indicates that, by discarding trees producing thick-hulled fruits or poorly filled nuts, a group of selected seed parents that was much better than average in oil content had been obtained.

Among the selections there are 24 individual trees that have borne fruits containing at least 25 percent of oil. Some of these trees have also produced high yields. Provided they transmit these characteristics to their progeny, either seedling or budded, they offer great promise for the tung industry.

The means for the different years are considered to come from independent samples, and the statistical significance of the differences

**TABLE 9.**—Frequency distribution of percentage of oil in 535 samples of whole equalized fruits from selected tung trees,<sup>1</sup> grouped according to crop year, 1938–40

Midelass value for oil in whole	Samples for indicated year of selection						
fruit (percent)	1938	1939	1940	1938–40			
6.0 7.0 8.0 9.0 10.0 11.0	Number 1 0 0 0 0 1 2	Number 0 0 0 0 0 0 0	Number 0 1 0 1 2	Number 1 0 1 0 1 3			
12.0 13.0 14.0 15.0 16.0 17.0 18.0	$2 \\ 3 \\ 4 \\ 4 \\ 11 \\ 14 \\ 26$	$ \begin{array}{c} 0 \\ 3 \\ 3 \\ 5 \\ 15 \\ 14 \end{array} $	$     \begin{array}{c}       1 \\       3 \\       4 \\       1 \\       3 \\       6     \end{array} $				
19.0 20.0 21.0 22.0 23.0 24.0 25.0	$ \begin{array}{c} 19 \\ 39 \\ 32 \\ 31 \\ 13 \\ 12 \\ 5 \end{array} $	$     \begin{array}{c}       20 \\       37 \\       20 \\       29 \\       22 \\       25 \\       13     \end{array} $		$47 \\ 89 \\ 72 \\ 72 \\ 43 \\ 43 \\ 22$			
26.0 27.0 28.0 Total	$\begin{array}{r} 3\\1\\0\\221\end{array}$	213	3 2 0 101	9 3 1 535			
Mean percent of oil	$19.9 \pm 0.2$	20. $8 \pm 0.2$	20. $0 \pm 0.4$	20. $3 \pm 0.1$			

<sup>1</sup> 1 sample was taken from each tree each year, but in a few instances samples were taken from the same tree in each of 2 or 3 different years.

Midclass value for kernel in	Samples for indicated year of selection						
whole fruit (percent)	1938	1939	1940	1938-40			
	Number	Number	Number	Number			
8.0	. 1	0	0	1			
10.0	- 0	0	Ő	Ō			
12.0	0	Ō	Ō	Ő			
14.0	0	Ő	Õ	. Ő			
16.0	. 0	Ō	Õ	Ő			
18.0	Ū Ū	Ō	Õ	- Ö			
20.0	0	0	3	3			
22.0	2	1	6	9			
24.0	3	5	8	16			
26.0	9	10	8	$\tilde{27}$			
28.0	26	24	5	55			
30.0	31	36	7	74			
32.0	48	48	23	119			
34.0	51	48	13	112			
36.0	29	29	15	73			
38.0	18	11	8	37			
40.0	2	1	2	5			
42.0	. 1	0	$\overline{2}$	3			
44.0	0	0	ō	Õ			
46.0	0	Ő	Õ	Ŏ			
48.0	- 0	Ŭ Û	1	1			
Total	221	213	101	535			
Mean percent of kernel	$32.3 \pm 0.3$	$32.0 \pm 0.2$	$\overline{31.4 \pm 0.6}$	$32.0\pm0.2$			

TABLE 10.—Frequency distribution of percentage of kernel in 535 samples of whole equalized fruits from selected tung trees,<sup>1</sup> grouped according to crop year, 1938–40

<sup>1</sup> 1 sample was taken from each tree each year, but in a few instances samples were taken from the same tree in each of 2 or 3 different years.

TABLE 11.—Frequency distribution of percentage of filling in 535 samples of nuts from selected tung trees,<sup>1</sup> grouped according to crop year, 1938–40

Midclass value for filling of	Samples for indicated year of selection					
nuts (percent)	1938	1939	1940	1938-40		
	Number	Number	Number	Number		
46.5	0	1	2	3		
49.5	0	. 0	1	1		
52.5	3	0	0	3		
55.5	1	2	2	5		
58.5	2	1	1	4		
61.5	2	2	1	5		
64.5	3	4	1	8		
67.5	6	5	3	14		
70.5	6	7	1	14		
73.5	9	8	2	19		
76.5	20	24	1	45		
79.5	19	34	8	61		
82.5	34	52	17	103		
85.5	43	37	36	116		
88.5	37	27	23	87		
91.5	28	8	2	38		
94.5	8	1	0	9		
Total	221	213	101	535		
Mean percent of filling	82. $6 \pm 0.6$	80. $9 \pm 0.5$	81. $9 \pm 0.9$	81. $8 \pm 0.4$		

<sup>1</sup> 1 sample was taken from each tree each year, but in a few instances samples were taken from the same tree in each of 2 or 3 different years.

has been calculated by means of Goulden's formula (4) for groups with different numbers of individuals. On this basis the mean oil content in 1939, 20.8 percent, was significantly greater than that in 1938, 19.9 percent, or that in 1940, 20.0 percent. This was true even though the trees bore a comparatively light crop in 1939 and there is a tendency sometimes for the fruits to develop thick hulls when the crop is light. Thick hulls tend to decrease the percentage of oil in the whole fruits. The relation of weather to oil content is not clear, but it seems reasonable that annual variations in precipitation, temperature, and sunshine may have a profound effect on oil synthesis.

To determine whether the greater oil content in 1939 was due to a larger percentage of kernel in the whole fruit, as might be the case if selections made in 1939 had thinner hulls than those made in the other 2 years, the samples were classified according to percentage of kernel in the fruit (table 10). It was found that the samples of fruits actually analyzed in 1939 had a lower average percentage of kernel than those analyzed in 1938; it necessarily follows that if the average oil content was higher in 1939 than in 1938, the percentage of oil in the kernel must have been considerably higher in 1939 than in 1938. This finding, involving results of different seasons, differs from that of Lagasse and Fisher (8), who, working with samples taken from a considerable number of individual seed parents, found that in any one season a significant positive correlation exists between percentages of kernel in the whole fruit and of oil in the kernel. Percentage of filling of the nuts was estimated by cutting a representative sample in median cross section and estimating by the aid of standard charts the extent to which the kernel filled the shell. The data obtained show that the high oil content of 1939 selections cannot be attributed to better filling (table 11).

#### NUMBER OF FRUITS PER CLUSTER

A knowledge of the tree characteristics associated with oil content of the fruit would facilitate the selection of trees for breeding purposes. The relation of oil content to characters evaluated in the field can be deduced from data presented in tables 12 to 17. When each tree was selected, the fruits were counted in 10 to 20 clusters taken at random and the mean number per cluster was then calculated and recorded. The data on oil content of 456 samples of fruits from trees grouped according to the mean number of fruits borne per cluster are shown (table 12). It is interesting that nearly half of the samples were from trees that bore less than 2 fruits per cluster. In casual observations one tends to notice the large clusters, and a tree may have many clusters of 3 to 4 fruits and yet on the average have less than 2 per cluster. Although clusters of 10 fruits or more are frequently observed in the orchards, trees that have a mean of 4 fruits or more per cluster occur on the average in only 1 case out of 10.

The mean oil content of the fruits from trees with a mean of five fruits or more per cluster was 18.2 percent, which is rather low. The differences between the oil content of the fruits in these large clusters and that of each of the other four groups are statistically significant at the 1-percent level. No other differences attained sta-

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TABLE 12.—Frequence	y distribution of	f percentage of	oil in 456 samples
of whole equalized	fruits from sele	cted tung trees.	grouped accord
ing to number of	fruits per cluste	r	

Midclass value for	Samples for indicated number of fruits per cluster						
(percent)	1.0-1.9	2.0-2.9	3.0-3.9	4.0-4.9	5.0+		
$\begin{array}{c} 7.0 \\ 8.0 \\ 9.0 \\ 10.0 \\ 11.0 \\ 12.0 \\ 13.0 \\ 14.0 \\ 15.0 \\ 16.0 \\ 17.0 \\ 18.0 \\ 19.0 \\ 20.0 \\ 21.0 \\ 22.0 \\ 23.0 \\ 24.0 \\ 23.0 \\ 24.0 \\ 24.0 \\ 25.0 \\ 26.0 \\ 27.0 \\ 28.0 \\ 27.0 \\ 28.0 \\ 20.0 $	$\begin{array}{c} Number \\ 1 \\ 0 \\ 1 \\ 1 \\ 3 \\ 2 \\ 2 \\ 2 \\ 2 \\ 4 \\ 9 \\ 18 \\ 31 \\ 36 \\ 38 \\ 37 \\ 15 \\ 12 \\ 4 \\ 5 \\ 1 \\ 1 \\ 1 \\ \end{array}$	$\begin{array}{c} Number \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 1 \\ 1 \\ 3 \\ 3 \\ 1 \\ 4 \\ 9 \\ 8 \\ 23 \\ 9 \\ 14 \\ 10 \\ 13 \\ 5 \\ 1 \\ 0 \\ 0 \\ 0 \\ \end{array}$	$\begin{array}{c} Number \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 2 \\ 3 \\ 5 \\ 11 \\ 6 \\ 4 \\ 10 \\ 5 \\ 6 \\ 9 \\ 12 \\ 5 \\ 1 \\ 1 \\ 0 \end{array}$	$\begin{tabular}{cccc} Number & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & $	Number 0 0 0 0 0 0 0 0 1 1 1 1 1 2 0 0 0 1 1 1 2 0 0 0 0		
Total	223	106	81	26	20		
Mean percent of oil	$20.3 \pm 0.2$	$20.5\pm0.3$	$20.3\pm0.4$	$21.0\pm0.6$	$18.2\pm0.9$		

<sup>1</sup> Samples were taken during 3 years, 1938–40. 1 sample was taken from each tree each year, but in a few instances samples were taken from the same tree in each of 2 or 3 different years.

tistical significance. The data in tables 13 and 14 show further that the fruits from large clusters have a low percentage of kernels and tend to be poorly filled.

#### SEASON OF MATURITY

The fruits of a seedling tung tree may mature in September, October, or November. When a tree was selected, its characteristic season of maturity of fruit was estimated as early, medium, or late. Data on the oil content (table 15) of fruits from 441 samples from selected trees are given in relation to their season of maturity. It may be observed that there were 148 samples from trees classified as early, 221 from those classified as medium, and 72 from those classified as late. These proportions are not necessarily representative of seedling or-

T	ABLE	13.—	Freque	ncy d	istrib	ution	of	percenta	ge of	kernei	l in	456
	samp	oles of	whole	equali	ized f	ruits	from	selected	tung	trees,1	grou	iped
	accor	rding i	to numi	ber of	fruit	s per	clust	er				

Midclass value for	Samples for indicated number of fruits per cluster						
(percent)	1.0-1.9	2.0-2.9	3.0-3.9	4.0-4.9	5.0+		
$\begin{array}{c} 8.0 \\ 10.0 \\ 12.0 \\ 12.0 \\ 14.0 \\ 14.0 \\ 16.0 \\ 20.0 \\ 22.0 \\ 22.0 \\ 22.0 \\ 22.0 \\ 22.0 \\ 24.0 \\ 30.0 \\ 32.0 \\ 30.0 \\ 32.0 \\ 34.0 \\ 33.0 \\ 34.$	$\begin{array}{c} Number \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 2 \\ 4 \\ 3 \\ 10 \\ 22 \\ 35 \\ 53 \\ 53 \\ 53 \\ 53 \\ 53 \\ 53$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c } \hline Number & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & $	Number 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0		
Total	223	106	81	26	20		
Mean percent of kernel	$31.9 \pm 0.3$	32. $4 \pm 0.4$	32. $3 \pm 0.4$	33. $6 \pm 0.7$	30. $3 \pm 1.0$		

<sup>1</sup>Samples were taken during 3 years, 1938–40. 1 sample was taken from each tree each year, but in a few instances samples were taken from the same tree in each of 2 or 3 different years.

chards, because early and medium trees were selected in preference to late ones. The large tung leaves drop after the first severe freeze and dropped fruits of late-maturing trees may be covered up, thereby increasing the cost of harvesting. However, fruits that mature late in the season presumably would have a longer period in which to store oil and might ultimately attain a relatively high oil content. Contrary to expectations the data in table 15 show that fruits that matured early had the highest oil content, 20.0 percent, and fruits that matured late had the lowest, 17.6 percent. The difference between the late and medium types is statistically significant, and the early type has a significantly greater oil content than the medium type.

Data in tables 16 and 17, respectively, show clearly that the later the season of fruit maturity the lower the percentage of kernel and the percentage of filling of the nuts. The percentage of kernel ranged from 33.1 for fruits that matured early to 30.3 for fruits that matured

TABLE	14Fre	quency o	listributi	on of p	ercentage	of filling	in 456	s sam-
ples	of nuts f	rom sele	cted tung	y trees,	grouped	according	to m	ımber
of fr	uits per d	cluster						

Midclass value for	Samples for indicated number of fruits per cluster						
(percent)	1.0-1.9	2.0-2.9	3.0-3.9	4.0-4.9	5.0+		
$\begin{array}{c} 46.5 \\ 49.5 \\ 52.5 \\ 55.5 \\ 61.5 \\ 61.5 \\ 64.5 \\ 67.5 \\ 70.5 \\ 70.5 \\ 79.5 \\ 82.5 \\ 85.5 \\ 85.5 \\ 88.5 \\ 91.5 \\ 94.5 \\ \end{array}$	$\begin{array}{c} Number \\ 2 \\ 0 \\ 0 \\ 1 \\ 2 \\ 1 \\ 2 \\ 3 \\ 7 \\ 6 \\ 12 \\ 222 \\ 39 \\ 63 \\ 43 \\ 16 \\ 4 \end{array}$	$\begin{matrix} Number & 2 \\ & 0 \\ & 2 \\ & 1 \\ & 0 \\ & 1 \\ & 0 \\ & 2 \\ & 3 \\ & 3 \\ & 14 \\ & 13 \\ & 26 \\ & 18 \\ & 14 \\ & 6 \\ & 1 \end{matrix}$	$\begin{array}{c} Number \\ 0 \\ 1 \\ 0 \\ 2 \\ 0 \\ 2 \\ 2 \\ 2 \\ 4 \\ 1 \\ 7 \\ 8 \\ 13 \\ 13 \\ 14 \\ 11 \\ 2 \\ 1 \end{array}$	$\begin{array}{c} Number \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 3 \\ 0 \\ 0 \\ 4 \\ 4 \\ 2 \\ 4 \\ 5 \\ 3 \\ 0 \\ 0 \\ \end{array}$	$\begin{array}{c} Number \\ 0 \\ 0 \\ 1 \\ 1 \\ 1 \\ 1 \\ 0 \\ 4 \\ 4 \\ 1 \\ 2 \\ 1 \\ 1 \\ 1 \\ 6 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0$		
Total	223	106	81	26	20		
Mean percent of filling	$83.0 \pm 0.5$	$80.7\pm0.9$	$79.4 \pm 1.0$	$81.2 \pm 1.6$	$72.2 \pm 2.3$		

<sup>1</sup> Samples were taken during 3 years, 1938–40. 1 sample was taken from each tree each year, but in a few instances samples were taken from the same tree in each of 2 or 3 different years.

late, and the percentages of filling for the early, medium, and late types were 82.4, 81.6, and 77.9, respectively. All differences in percentages of kernel and of filling are statistically significant except the difference in filling of early and medium types, 0.8 percent.

In view of the increased difficulty in harvesting the fruits and of the decrease in the oil content, trees bearing late-maturing fruits should be used as parents only if exceptionally desirable for some other important characteristic, such as yield.

### SIZE OF LEAVES

Early observations led to the belief that trees with large leaves were usually less vigorous than those with small ones. Some of the trees from which samples of fruit had been analyzed for oil content were classified according to estimated leaf size, and the samples were grouped in these classes (table 18). As shown, the size of the leaves is not correlated with the percentage of oil in the whole fruits. For example, samples from trees with small leaves had a mean of 20.6 percent oil and those from trees with very large ones 20.3 percent. There is no statistically significant difference between any two of the means in table 18.

Midclass value for oil in whole fruit	Samples for indicated season of maturity			
(percent)	Early	Medium	Late	
$\begin{array}{c} 6.0 \\ 7.0 \\ 7.0 \\ 8.0 \\ 9.0 \\ 9.0 \\ 10.0 \\ 10.0 \\ 11.0 \\ 12.0 \\ 13.0 \\ 14.0 \\ 15.0 \\ 14.0 \\ 15.0 \\ 14.0 \\ 15.0 \\ 10.0 \\ 1$	$\begin{array}{c} Number \\ 1 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 1 \\ 1 \\ 1 \\ 3 \\ 1 \\ 6 \\ 9 \\ 9 \\ 9 \\ 24 \\ 21 \\ 31 \\ 11 \\ 17 \\ 8 \\ 3 \\ 1 \end{array}$	$\begin{array}{c} Number \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 3 \\ 2 \\ 2 \\ 2 \\ 5 \\ 9 \\ 15 \\ 18 \\ 25 \\ 35 \\ 29 \\ 27 \\ 24 \\ 17 \\ 7 \\ 2 \\ 1 \end{array}$	Number 0 1 0 0 0 0 4 6 1 1 2 4 9 9 15 7 7 7 2 0 0 1 2 0	
Total	148	221	72	
Mean percent of oil	20. $0 \pm 0.2$	19. $3 \pm 0.2$	17. $6 \pm 0.4$	

 TABLE 15.—Frequency distribution of percentage of oil in 441 samples
 of whole equalized fruits from selected tung trees,<sup>1</sup> grouped according to season of maturity

<sup>1</sup>Samples were taken during 3 years, 1938–40. 1 sample was taken from each tree each year, but in a few instances samples were taken from the same tree in each of 2 or 3 different years.

# SUMMARY AND CONCLUSIONS

Moisture content of tung fruits can be determined by drying whole fruits, but the most rapid and satisfactory method is to grind a 25fruit sample, mix thoroughly, and determine moisture in duplicate 5-gm. portions at 100° C. and 4-mm. pressure over phosphorus pentoxide.

Moisture in tung kernels can be determined by grinding or flaking and drying them at 70° to 100° C. and 4-mm. pressure.

Oil in kernels can be determined readily by flaking the kernels to a thickness of about  $50\mu$  with a mechanical flaker and extracting the oil in a Goldfisch unit. More than 99 percent of the total oil can be extracted in 12 hours without regrinding the residue. Tung kernels must be flaked longitudinally because the micropylar end contains less oil than the midsection.

The oil can be determined either by direct weighing after the solvent is driven off or by obtaining the difference in weight of the kernels before and after extraction. Rapid weighing of the residue is es-

Midclass value for kernel in whole fruit	Samples for indicated season of maturity			
(percent)	Early	Medium	Late	
	Number	Number	Number	
8.0	. 1	0	0	
10.0	0	0	0	
12.0	0	0	0	
14.0	0	0	0	
16.0	0	0	0	
18.0	0	0	0	
20.0	0	2	.1	
22.0	1	2	2	
24.0	1	2	7	
26.0	2	17	7	
28.0	10	26	7	
30.0	18	29	14	
32.0	32	52	16	
34.0	44	45	8	
36.0	20	31	7	
38.0	15	13	3	
40.0	3	1	0	
42.0	· 0	1	0	
44.0	0	0	. 0	
46.0	0	0	0	
48.0	1	0	0	
Total	148	221	72	
Mean percent of kernel	$33.1 \pm 0.3$	$31.9 \pm 0.3$	$30.3 \pm 0.5$	

TABLE 16.—Frequency distribution of percentage of kernel in 441 samples of whole equalized fruits from selected tung trees,<sup>1</sup> grouped according to season of maturity

<sup>1</sup> Samples were taken during 3 years, 1938–40. 1 sample was taken from each tree each year, but in a few instances samples were taken from the same tree in each of 2 or 3 different years.

TABLE 17.—Frequency distribution of percentage of filling in 441 samples of nuts from selected tung trees,<sup>1</sup> grouped according to season of maturity

Midelass value for filling of nuts (percent)	Samples for indicated season of maturity				
indenies value for himnig of nuce (percent)	Early	Medium	Late		
	Number	Number	Number		
46.5	0	2	1		
49.5	. 0	0	1		
52.5	0	3	0		
55.5	1	2	2		
58.5	3	1	0		
61.5	1	0	2		
64.5	2	3	4		
67.5	6	3	4		
70.5	2	7	3		
73.5	5	7	4		
76.5	13	20	5		
79.5	12	27	12		
82.5	26	45	14		
85.5	36	52	9		
88.5	27	35	8		
91.5	10	12	3		
94.5	4	2	0		
Total	148	221	72		
Mean percent of filling	$82.4 \pm 0.6$	81. $6 \pm 0.5$	77. $9 \pm 1.2$		

 $^1$  Samples were taken during 3 years, 1938–40. 1 sample was taken from each tree each year, but in a few instances samples were taken from the same tree in each of 2 or 3 different years.

TABLE 18.—Frequency distribution of percentage of oil in 333 samples of whole equalized fruits from selected tung trees,<sup>1</sup> grouped according to leaf size

Midclass value for oil	Samples for indicated leaf size						
in whole fruit (percent)	Very small	Small	Medium	Large	Very large		
8.0         9.0         10.0         11.0         12.0         13.0         14.0         15.0         16.0         17.0         18.0         19.0         20.0         21.0         22.0         23.0         24.0         25.0         26.0         27.0	$\begin{array}{c} Number \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 2 \\ 0 \\ 2 \\ 3 \\ 4 \\ 8 \\ 6 \\ 8 \\ 5 \\ 2 \\ 1 \\ 1 \\ 0 \\ \end{array}$	$\begin{array}{c} Number \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 1 \\ 2 \\ 1 \\ 6 \\ 4 \\ 2 \\ 10 \\ 8 \\ 10 \\ 5 \\ 5 \\ 4 \\ 1 \\ 0 \end{array}$	$\begin{array}{c} Number \\ 1 \\ 0 \\ 1 \\ 0 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2$	$\begin{array}{c} Number \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 1 \\ 0 \\ 0 \\ 2 \\ 4 \\ 5 \\ 11 \\ 2 \\ 7 \\ 6 \\ 9 \\ 3 \\ 2 \\ 0 \\ \end{array}$	Number 0 0 1 1 2 2 3 3 1 1 2 2 9 9 9 9 9 9 4 4 1 7 7 1 2 0		
Total	43	60	131	53	46		
Mean percent of oil	$20.6 \pm 0.4$	$20.6 \pm 0.4$	$20.5\pm0.3$	$21.2\pm0.4$	$20.3\pm0.5$		

<sup>1</sup>Samples were taken during 3 years, 1938-40. 1 sample was taken from each tree each year, but in a few instances samples were taken from the same tree in each of 2 or 3 different years.

sential because of its hygroscopic properties. Determinations by loss in weight of residue are simpler and more expeditious and are adequate for most purposes.

When tung kernels have not more than 5 percent of moisture the oil whether extracted with acetone, ethyl ether, or petroleum ether is practically the same in amount and in quality.

The fruits of some seedling trees are more variable in oil content than those of others. In nearly all instances the degree of precision attained by using a 25-fruit sample is such as to permit distinguishing 2 samples differing in oil content of the whole fruit by not less than 2.00 percent.

Whole fruits of seedling trees selected during 1938, 1939, and 1940 ranged from 6.0 to 28.0 percent in oil content when the moisture content had been equalized, that is, brought to equilibrium with air over a saturated solution of calcium chloride at a room temperature of approximately 25° C. The average oil content was highest in 1939, 20.8 percent, and lowest in 1938, 19.9 percent.

Wide variations in oil content existed among the fruits of the tung trees studied. The best oil content is to be expected in early-maturing fruits borne in clusters of four or less. Thin-hulled fruits with well-filled nuts tend to have good oil content. However, an actual chemical analysis is the only satisfactory criterion of oil content for use in breeding and selection.

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